Allo Project Report

# Summary

The solubility of Allopregnanolone (Allo) was evaluated in various organic solvents and excipient-based formulations to improve its dissolution in aqueous phosphate-buffered saline (PBS). The investigation screened Dichloromethane (DCM), T-Butanol, Ethanol, and Isopropyl Alcohol as solvents and Polysorbate 80, Span 20, Span 80, and Phosphatidylcholine (PPTC) as excipients.

Solvent screening identified DCM, Ethanol, and Isopropyl Alcohol as effective solvents, achieving Allopregnanolone concentrations up to 100 mg/mL, 6.25 mg/mL, and 6.25 mg/mL, respectively; Acetone, HCl, and Citric Acid were unsuitable. However, creating stable aqueous formulations with the tested excipients was unsuccessful. Allopregnanolone consistently precipitated from solution upon organic solvent removal and addition of PBS, regardless of excipient concentration or pH. Although Span 20 showed potential for solubilization in one instance, the result was not reproducible. Quantitative analysis revealed varied solubility enhancement in organic solvents, with Span 80 in T-Butanol yielding the highest value (127.0 mg).

Data reproducibility was affected by methodological challenges, including inconsistent solvent removal, material splashing, caking, and chemical incompatibility between DCM and polystyrene labware. Allopregnanolone exhibited poor solubility in the tested aqueous formulations. Further investigation is required to identify more effective excipients and to establish a robust, reproducible methodology for preparing stable solutions.

# Introduction

The limited solubility of the neurosteroid Allopregnanolone constrains the development of stable and administrable pharmaceutical formulations. This investigation systematically evaluated strategies to enhance its solubility.

A baseline assessment established Allopregnanolone's solubility in Dichloromethane (DCM), T-Butanol, Ethanol, and Isopropyl Alcohol. Subsequently, a range of excipients were evaluated for their ability to enhance and maintain solubility in an aqueous buffer (PBS) following organic solvent removal. The tested excipients included surfactants (e.g., Polysorbate 80, Tween 20, Span 20, Span 80), lipids (e.g., Phosphatidylcholine, Castor Oil), and other formulation aids (e.g., PEGs, Dextrans, amino acids).

Formulations were prepared in microplates or vials, and the organic solvent was removed via heating. This process was complicated by procedural issues, including material splashing and caking. Allopregnanolone precipitated in the majority of tested formulations, indicating a persistent challenge in achieving a stable, solubilized state.

# Objectives

Based on the context provided, the project had the following objectives:

1. To determine the solubility of Allopregnanolone in various organic solvents, such as Dichloromethane (DCM), T-Butanol, Ethanol, and Isopropyl Alcohol.

2. To evaluate the ability of numerous excipients (e.g., Polysorbates, Spans, Phosphatidylcholine) to enhance the solubility of Allopregnanolone.

3. To assess the stability of Allopregnanolone in different formulations by observing its tendency to precipitate under various conditions, including different excipient combinations, concentrations, and pH levels.

4. To prepare a standard dilution series of the drug to establish a concentration-response curve for subsequent assays.

# Methodology

## Materials

Allopregnanolone was the active pharmaceutical ingredient (API). Solvents included Dichloromethane (DCM), Tert-Butanol (T-Butanol), Ethanol, Isopropyl Alcohol, and Acetone. The aqueous phase was 1X Phosphate-Buffered Saline (PBS), adjusted to pH 6.5, 7.5, or 10.0 for specific experiments. Evaluated excipients included Phosphatidylcholine (PPTC), Polysorbate 80 (P80), Span 20, Span 80, Tween 20, Tween 40, Castor Oil, Cholesterol, Corn Oil, Cottonseed Oil, Soybean Oil, L-Arginine, L-Cysteine, Dextran 40, Glycine, and various Polyethylene Glycols (PEGs). Experiments were conducted in glass vials, Nunc polystyrene 96-well plates, and Costar polypropylene 96-well plates.

## Allopregnanolone Solubility Screening

Baseline Allopregnanolone solubility was determined per internal procedure WID-001. Weighed quantities of Allopregnanolone were dispensed into vials, and solvent was added incrementally to target concentrations from 6.25 mg/mL to 100 mg/mL. After each solvent addition, mixtures were mixed for 2 minutes and visually assessed for complete dissolution to determine the saturation point.

**Table 1: Allopregnanolone Solubility in Various Organic Solvents**

|  |  |
| --- | --- |
| Solvent | Determined Solubility (mg/mL) |
| Dichloromethane (DCM) | 100 |
| Tert-Butanol | 25 |
| Ethanol | 6.25 |
| Isopropyl Alcohol | 6.25 |
| Acetone | < 6.25 |
| HCl | < 6.25 |
| Citric Acid | < 6.25 |

## Preparation of Stock Solutions

Allopregnanolone and excipient stock solutions were prepared in DCM or T-Butanol. Allopregnanolone was prepared at 10 mg/mL or 12 mg/mL. Excipients were prepared at various concentrations (typically 10 mg/mL or 25 mg/mL) by dissolving a weighed mass of the compound in a calculated volume of the appropriate organic solvent.

**Table 2: Preparation of Allopregnanolone and Excipient Stock Solutions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | Solvent | Mass (mg) | Solvent Volume (mL) | Target Concentration (mg/mL) |
| Allopregnanolone | DCM | 85.50 | 8.55 | 10 |
| Allopregnanolone | T-Butanol | 81.50 | 8.15 | 10 |
| Phosphatidylcholine | DCM | 106.2 | 4.25 | 25 |
| Polysorbate 80 | DCM | 80.1 | 3.20 | 25 |
| Span 80 | DCM | 94.1 | 3.76 | 25 |
| Tween 20 | DCM | 82.0 | 3.28 | 25 |
| Phosphatidylcholine | T-Butanol | 87.9 | 3.52 | 25 |
| Polysorbate 80 | T-Butanol | 109.9 | 4.40 | 25 |
| Span 80 | T-Butanol | 127.0 | 5.08 | 25 |
| Tween 20 | T-Butanol | 94.2 | 3.77 | 25 |

## Excipient Screening for Solubility Enhancement

To evaluate the ability of excipients to maintain Allopregnanolone solubility in an aqueous environment, organic solutions of Allopregnanolone and an excipient were combined with an aqueous PBS solution, followed by removal of the organic solvent via evaporation.

Test formulations were prepared by combining 1 mL of Allopregnanolone stock (10 mg/mL in DCM or T-Butanol) with 1 mL of an excipient stock (25 mg/mL in the same solvent), followed by the addition of 1 mL of 1X PBS. Control vials containing only the drug or only the excipient were also prepared. Vials were continuously mixed in a fume hood to evaporate the organic solvent and then visually inspected for precipitation.

Excipient stock solutions were prepared in PBS, T-Butanol, or DCM ([TABLE\_3]). For organic-soluble excipients, 50 µL of Allopregnanolone stock (12 mg/mL) was combined with 50 µL of the corresponding excipient stock in a well, followed by 100 µL of 1X PBS, yielding a final Allopregnanolone concentration of 6 mg/mL prior to evaporation. For aqueous-soluble excipients, 100 µL of Allopregnanolone stock was mixed with 100 µL of the excipient-PBS solution. Plates were heated at 70°C with gentle shaking to evaporate the organic solvent (~16 min for DCM, ~32 min for T-Butanol). Initial experiments with DCM in Nunc polystyrene plates resulted in well degradation; subsequent DCM experiments were performed in Costar polypropylene plates. Precipitation was assessed by measuring absorbance at 600 nm and 860 nm on a Tecan plate reader before and after heating.

**Table 3: Excipient Stock Solution Concentrations for High-Throughput Screening**

|  |  |  |
| --- | --- | --- |
| Solvent System | Excipient | Concentration (mg/mL) |
| PBS | L-Arginine, L-Cysteine, Dextran 40, Dextran 60-90, Glycine, PEG 300, PEG 3350, PEG 400 | 50 |
| PBS | L-Histidine, Beta-Cyclodextrin, Gamma-Cyclodextrin | 10 |
| PBS | Poloxamer 188, Polysorbate 80, Tween 20, Dexolve | 25 |
| PBS | Soluplus, Span 20 | 6.25 |
| PBS | Tween 40 | 12.5 |
| T-Butanol | Castor Oil, Corn Oil, Cottonseed Oil, Soybean Oil | 25 |
| T-Butanol | PEG 300, PEG 400 | 50 |
| T-Butanol | Cholesterol | 0.625 |
| T-Butanol | PPTC, Polysorbate 80, Span 20, Tween 20, Tween 40 | 10 |
| DCM | Castor Oil, Corn Oil, Cottonseed Oil, Soybean Oil | 50 |
| DCM | Cholesterol | 20 |
| DCM | PPTC, Polysorbate 80, Span 20, Tween 20, Tween 40 | 10 |

**Table 4: Plate Configuration for T-Butanol Excipient Screening**

|  |  |
| --- | --- |
| Plate Row | Wells 1-12 Content |
| A, D | 50 µL Excipient/T-Butanol + 50 µL Allo/T-Butanol |
| B, E | 50 µL Excipient/T-Butanol + 50 µL T-Butanol (Excipient Control) |
| C, F | 50 µL Allo/T-Butanol + 50 µL T-Butanol (Drug Control) |

**Table 5: Plate Configuration for DCM Excipient Screening**

|  |  |
| --- | --- |
| Plate Row | Wells 1-10 Content |
| A, D | 50 µL Excipient/DCM + 50 µL Allo/DCM |
| B, E | 50 µL Excipient/DCM + 50 µL DCM (Excipient Control) |
| C, F | 50 µL Allo/DCM + 50 µL DCM (Drug Control) |

Formulation parameters for promising excipients were optimized in vials. The effect of Allopregnanolone concentration was tested by combining 0.5 mL of Span 20 or Span 80 stock (10 mg/mL in DCM) with 0.5 mL of Allopregnanolone stock at 6, 8, 10, or 12 mg/mL in DCM. The impact of pH was studied using a formulation of Allopregnanolone (6 mg/mL) and Span 20 (5 mg/mL) with PBS at pH 6.5 and 10.0. Excipient combinations were also screened ([TABLE\_6]). In all cases, 100–200 µL of PBS was added, and vials were heated at 70°C to remove DCM before visual assessment.

**Table 6: Formulations for Excipient Combination Screening**

# Results

## Allopregnanolone Solvent Solubility Screening

The solubility of Allopregnanolone was determined in various organic solvents using a serial dilution format from a starting concentration of 100 mg/mL. Allopregnanolone was highly soluble in dichloromethane (DCM) at 100 mg/mL and soluble in tert-Butanol (T-Butanol) at 25 mg/mL. Solubility was lower in ethanol and isopropyl alcohol, with complete dissolution observed at 6.25 mg/mL. Allopregnanolone was insoluble in acetone, HCl, and citric acid at all tested concentrations (6.25–100 mg/mL).

**Table 1.\*\* Summary of Allopregnanolone solubility in various solvent**

|  |  |
| --- | --- |
| Solvent | Maximum Observed Solubility (mg/mL) |
| Dichloromethane (DCM) | 100 |
| tert-Butanol (T-Butanol) | 25 |
| Ethanol | 6.25 |
| Isopropyl Alcohol | 6.25 |
| Acetone | < 6.25 |
| HCl | < 6.25 |
| Citric Acid | < 6.25 |

## Excipient Screening in Vials with Solvent Evaporation

Four excipients—Phosphatidylcholine (PPTC), Polysorbate 80 (P80), Span 80, and Tween 20—were screened for their ability to enhance Allopregnanolone solubility in an aqueous environment. Stock solutions of Allopregnanolone (10 mg/mL) and excipients (25 mg/mL) in DCM or T-Butanol were combined in glass vials with 1X PBS. The organic solvents were removed via continuous mixing in a fume hood. This evaporation method resulted in significant splashing and caking of material on the vial walls, leading to inconsistent final PBS volumes.

# Conclusion

Attempts to increase the aqueous solubility of allopregnanolone in PBS using various excipients and organic co-solvents had limited success. Allopregnanolone showed high solubility in organic solvents such as DCM (100 mg/mL) and T-Butanol (25 mg/mL), but this did not translate to stable aqueous formulations after solvent removal. Of the excipients evaluated—including phosphatidylcholine, polysorbate 80, and Tween 20—only Span 20 partially dissolved allopregnanolone in the final PBS buffer following DCM evaporation. In nearly all other cases, including combinations with Span 80 and various oils, allopregnanolone precipitated out of solution.

Methodological challenges compromised experimental reproducibility. Polystyrene plates were incompatible with DCM, dissolving the wells and requiring experiments to be repeated in polypropylene plates. The solvent evaporation process caused significant splashing and caking, which resulted in inconsistent final PBS volumes.

A stable 6 mg/mL aqueous allopregnanolone formulation was not achieved; however, Span 20 was identified as the most promising excipient for future development. Further experimentation is required to quantify the solubility limit with Span 20 and to establish a more accurate and reproducible method for solvent removal.